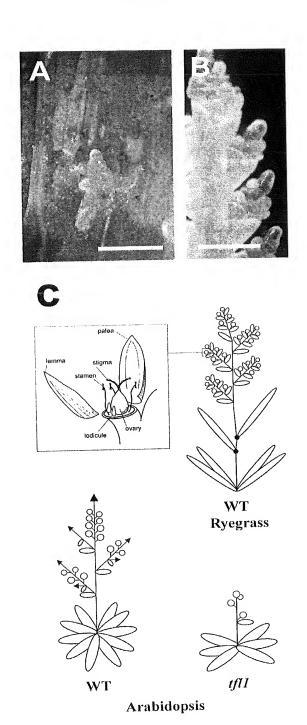
Docket No.: 0147-0262PUS1

App No.: 10/507,355 Docket No.: 0147-0262PU Inventor: Klaus K. NIELSEN et al. Title: METHOD OF REPRESSING FLOWERING IN A PLANT

REPLACEMENT SHEET

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# FIGURE 1



REPLACEMENT SHEET

App No.: 10/507,355 Docket No.: 0147-0262PUS1

Inventor: Klaus K. NIELSEN et al.

Title: METHOD OF REPRESSING FLOWERING IN A PLANT

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|     | GCC   | -76 |
|-----|---|-----|
| -75 | ${\tt CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCTCTCCAACC}$   | -1  |
| 1   | ${\tt ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTCTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTGTG$  | 75  |
| 76  | ${\tt AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT}$   | 150 |
| 151 | ${\tt AAACCAAGAGTAGAGGTTCAGGGGGGTGACTTGCGATCCTTATTCACATTGGTTATGACGGACCCAGATGTGCCA}$   | 225 |
| 226 | $\tt GGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATGATTATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCACTGGATGTCAGTAATATACCTTGGGACAACAGATGCTTCACTGGATGTCAGTAATATACCTTGGGACAACAGATGCTTTCACTGGATTGTCAGTAATATACCTTGGGACAACAGATGCTTCACTGGATGATATATACCTTGGGACAACAGATGCTTCACTGGATGTCAGTAATATACCTTGGGACAACAGATGCTTCAACTAGATGATATACCTTGGGACAACAGATGCTTTCACTGGATTGTCAGTAATATACCTTGGGACAACAGATGCTTTCACTGGATGATATATACCTTGGGACAACAGATGCTTTCACTGGATAATATACCTTGGGACAACAGATGCTTTCACTGGATGATATATACCTTGGGATGATATATACCTTGGATATATACCTTGGATATATAT$ | 300 |
| 301 | $\tt TTTGGGGGGGGGGGTCATGAGCTATGAGAGCCCAAAGCCCAACATTGGAATCCACAGGTTCATTTTTGTGCTCTTC$  | 375 |
| 375 | ${\tt AAGCAGAAGCGAAGGCAGACTGTATCTGTGCCTTCCTTCAGGGATCATTTCAACACCCGCCAGTTTGCTGTGGAT}$   | 450 |
| 451 | ${\tt AATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAG$  | 525 |
| 526 | ${\tt TCGAGTTCTTGGCTATCCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTTTCT$  | 600 |
| 601 | ${\tt CCTCTTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGATCAAATAAAT$   | 675 |
| 675 | ${\tt GCATTTTGGAGATTGTATTAGATTATTTGTAAGCAGTGAGATCAGCAACCATGTGTTAACATAAGCCAGTACAT}$  | 750 |
| 751 | ${\tt TAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAG$   | 825 |
| 026 | አ አ ርጥር/ርር እ አ አ አ አ አ አ አ አ አ አ አ አ አ አ አ አ አ አ  | 851 |

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# **FIGURE 3A**

| -3600 | ${\tt cactagtaacggccgccagtgtgctggaattcagggtaatacgactcactatagggmgctcgaggatcttcccac}$  | -3526 |
|-------|--|-------|
| -3525 | ${\tt cagtgtgcattcatgtgttacttaccactctccaacttgagggactcaagattggtgggcggctccttttcgctg}$  | -3451 |
| -3450 | a agegate cca a aggt g teggg ta ae ggt ta t ga cag ca aa cag aa aa ca tege ca tet g ca egga ag ce aga ag tegga ag cag aa gega ag cag aa gega ag cag aa gega ag cag aa gega ag cag ag c | -3376 |
| -3375 | $agttactat \verb gtcaaagggatataaaaaactcactaatgaagggggatgctattgctgagataaactgctatctca $  | -3301 |
| -3300 | tetacaggtgagattgeaagtatacttgacaacagggccagatggtatggcatgaagaaaattagggctggagta  | -3226 |
| -3225 | gaa aggta agatat gcat ggat t t ggat gagat ggctag agg t t gcgagat at caaa t agaa gacact t ctt caact gagat gagat at caaa t agaa gacact t ctt caact ggat gagat at caaa t agaa gacact t ctt caact ggat gagat gagat gagat at caaa t agaa gacact t ctt caact ggat gagat gaga | -3151 |
| -3150 | at gattca at a gaag at gcat gt gccatta cag a g t gattattat gtccttttta aa gag at gcttac gtccct  | -3076 |
| -3075 | $\tt gacctttcctataacacaattacactcctttgctagacttttcctgctataattgtctttcctcgccaaaagaat$  | -3001 |
| -3000 | a a tactataga a cttccta attta atttcccctt attttcttggactct atctta attctcctcct attgttcag  | -2926 |
| -2925 | $\tt ccaaggactgctccttccatttacttgcgccacgggctgactga$   | -2851 |
| -2850 | $\verb cctgaatctatttctcacctcatgctgcaatgctccttctcacagcaaatatggtatgatatctgcagtaagctc \\$   | -2776 |
| -2775 | a a ccttctgccatgtatgccagttggcaacgccgagttcagcatttggttcgccgccagctgccgccaacgctcaa   | -2701 |
| -2700 | $\tt ccagccctgcagaagggtgctaaatccatcatccttactctctggagattatggaagacgaggaacgatgct$   | -2626 |
| -2625 | $at \verb cttcaaaaatctggccccaacagactcgccttagttcagtcgatcctagatgaagcctgtcaatggtcgtta $   | -2551 |
| -2550 | $\tt gccggtgctaaggcgctacgtcagttacctttacatgctagaccccctgatgttagccttgatgaggaactctag$  | -2476 |
| -2475 | $\tt gtctaactaagttagccctgtacagtttttttttctctttttcctttttctttttttt$   | -2401 |
| -2400 | $\verb tttggtagctttgetactcttgtatgctcccgtcttctcgacggcttcttctaatatataatgacgcatgctttg $   | -2326 |
| -2325 | $\tt gcatgtgttcgagaaaaaaatttacttacctcttaggctatattctcttcaccaacttggactccacaaagcttc$  | -2251 |
| -2250 | a a tege caact t g tee a aget g et g et g et g et g te et t te e a t g e a tee a t g e a t e a t g e a t e a t g e a t e a t g e a t e a t g e a t e a t g e a t e a t g e a t e a t e a t g e a t e | -2176 |
| -2175 | ${\tt catacca} aa aa aa aa aa gcta at gccgccct gtt gtttca aa t gaat tatct gatt gt gat gct gcta at ctttt$   | -2101 |
| -2100 | gcatatgagtctcgggcatatgaatgaacttggtttggcagaatgaaacaagagaggacttcttgatggatatag  | -2026 |
| -2025 | cactgg taagetg aagt tetg tg ag eagget at gatg tt cecetg tt aaaaaaaaa aag getatg aaaaaet tg tg at the contract of the contrac | -1951 |
| -1950 | aggtgttaagtattggttttattttgcgtgcaaattggtatgcatggaaagttgtagtgctactagtctgtggtg  | -1876 |
| -1875 | $\verb ctactgtgctaccaacactgtagcactgccaaaaatttatgaaaaagtctgaacagacgagatgtatctatc$   | -1801 |
| -1800 | $\verb attcatggacccattttgttataattttcttttaaaataaaaaattccgtaaagaatcaataagtggaattattg $   | -1726 |
| -1725 | gaaatgaaaaaagtaaccaaaatactaaactttttttcaaatacagatcggatatcatggagacacactggctac  | -1651 |
| -1650 | $\verb cattggttggaatagctactagattccactacagctaggtgtcaagcaactataatggcatcagaatggagcaga \\$   | -1576 |
| -1575 | a a a a t g t caca ag c t g t a c t t c a c t c t ag c t g caca a a t g t c a ag c ag g c a t g a t t g cac t ag a c c a c a c c a c c a c c a c c a c c c a c c c a c c c a c   | -1501 |
| -1500 | aga a catag ta at g cata a ag ct g ta at t g g ct c cacta ct t at g g a a ac g a aga a at ct at t at   | -1426 |
| -1425 | $\tt aatcgagatgaagctgtgataattttatcgctgaaatgacatttcagcactagacagcaccctagacaattaagt$  | -1351 |
| -1350 | $\tt ggtggtggcactgtattccattcctttattctcttccatggtgtgttcccatagtactacaaagaagagaataaa$  | -1276 |
| -1275 | cagata a taatgg taatgcact tggg tatcg aag ttttagg aaa gattctaattctag ag caattgaactcaaca   | ~1201 |
| -1200 | a caacttcccttttccttaacagaaaaagaatcggtcaaacgaggcttgcctaaaccaacaacaactataaagacg  | -1126 |
| -1125 | a a catttg agggtg aag aggcttc cacgtg gacagtg ccg catgtttc tgtccactag at aacaccta aataata   | -1051 |
| ~1050 | gttaaaaaaacaagaggataagaatatcagaaagccagaccttaaatttctgcaagcaa  | -976  |
| -975  | ${\tt caaaaacgaattgatagtttaggaaagcatcactccaaagtgttttattcccgttctttttcatttgctccacaa}$  | -901  |
| -900  | $\tt gggcatacttcctaaatttctgcgaacaattacatctagatctttttaaaactgaagtattttagcatgaaaacg$  | -826  |
| -825  | cattgttctgtaatgtggctgtgaatttcggactgctcatctgatttccctctggtagaatacataaattat   | -751  |
| -750  | a caca a cag cat gata a t g t g caa a a c taa g cat caa a a t c t g caca t t g t cat g cag a a a c t a g g a cag g a g a caca t g t cat g cag a a c t a g g a cag g a g a caca t g t cat g caca t g t cac | -676  |
| -675  | $\tt ccagcactttgtcgtttgtcctaaccaatattaacatagttcagcaacataatcttcagagacccactagcatga$  | -601  |
| -600  | aggtgtgttatgtttcctaaagaaataacatgtaggtagtgatctacaataccttttttggggactataaggtgg  | -526  |
| -525  | $\tt gaaaccatcaacttgaaaaggtttccatttaatcaagtaaaaaaaa$   | -451  |
| -450  | ttaaaacagaatagagatatactaacaatgaaaatcaaacagttgtgcaaattgtatttatcgtagttagt  | -376  |
| -375  | ${\tt catgtttctggtgaaaaaattctctgcccctagaacttggaagaagatgcatgaagtattactccaaactccaac}$  | -301  |
| -300  | ${\tt actgtgcaactgatagaaaaaaaaaaaaaaaaaaaaaaa$   | -226  |
| -225  | $\tt ccttttcagttctttccacgcatacccaaccaaaaaaaaa$   | -151  |
| -150  | $\tt gcttacactcgaagcaggcttcttgcctctataagtagaggctcgtcgtactctagcaatgctcagtaagca{\tt GCC}$  | -76   |
| -75   | CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCTCTCCAACC  | -1    |
| 1     | ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTGTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTG  | 75    |
| 76    | AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT  | 150   |
| 151   | <b>AAACCAAGAGTAGAGGTTCAGGGGGGTGACTTGCGATCCTTATTCACATTG</b> gtagaatgcactcgactcgatctt  | 225   |
| 226   | ggaactccatattcaacttcgagtattgtatgcttgttttcttctttcgcagtggccataattattcatatttca  | 300   |
| 301   | gGTTATGACGGACCCAGATGTGCCAGGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGtaacctttctc  | 375   |
| 375   | atgcacagttttttctgctgggtggctactaagcacctaaatatattagtatatttttttgaaaggaaaatatat  | 450   |
|       |  |       |

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#### FIGURE 3B

| 451  | tagtatatgttgctaaggaatatagaagtacatcttcttcttgcacatatatagacagagagactattttaatag                     | 52   |
|------|---|------|
| 526  | $\verb cacttctaacgagagtcatttaccaataccttttacacttacacagg \verb attgtcagtaatatatacctgggacaacagg  $ | 60   |
| 601  | ATGCTTCATTTGG taggtccttctctgagatttgaattggtatattctatgttctgcattttgaatgaa                          | 679  |
| 675  | $\verb ctgaccttttgaattgcagg  \textbf{GGGGGGGGGTCATGAGCTATGAGGCCCAAAGCCCAACATTGGAATCCACAGGTTC} $ | 750  |
| 751  | ${\tt ATTTTGTGCTCTTCAAGCAGAAGCGAAGGCAGACTGTATCTGTGCCTTCCTT$                                     | 82   |
| 826  | ${\tt CAGTTTGCTGTGGATAATGATCTTGGCCTCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAG$                        | 900  |
| 901  | ${\tt AGGAGGCGCTGAAAATCGAGTTCTTGGCTATCCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTT}$             | 975  |
| 976  | ${\tt TCTGAAGTCAATGCTCCTCTTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGA}$             | 1050 |
| 1051 | ${\tt TCAAATAAATCAAGTGCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTTAA}$             | 1129 |
| 1126 | ${\tt CATAAGCCAGTACATTAGCAGGTCCATGTTTATGGTTTCATGTTGTTGTAAGCAGTTATCACTAGAAGGAAG$                 | 1200 |
| 1201 | CAGGTAGACAACCCAAACTGGCAAAAAAAAAAAGCTTTATCTA ctgtatggcccttgccggcttgatgttccatgc                   | 1275 |
| 1276 | accttttctgacatgctgtctactgtatgccaccgccactataatgtatgagatatgaatataaaatggagatat                     | 1350 |
| 1351 | ${\tt ccaaaatatccagatgattgcccactaaatgctaaatgtacatagtgggttttccacctattttgacttcatcat}$             | 1425 |
| 1426 | $\tt gtccttacacaaaatcagaaaacatccatttcatgcacattgatgcacactgcatattaacaatctattcagatt$               | 1500 |
| 1501 | tggctgtaaacacacccttattttccgcatccattaatattatattatgtaccctggacaggttaagcttttgcag                    | 1575 |
| 1576 | cacagtaagtaaccggatgaaattacaatatgatcctcgagcgccctat   | 1624 |

| 1   | ${	t MSRSVEPLIVGRVIGEVLDPFNPCVKMVATYNSNKLVFNGHELYPSAVVSKPRVEVQGGDLRSLFTLVMTDPDVP}$  | 75  |
|-----|---|-----|
| 76  | ${\tt GPSDPYLREHLHWIVSNIPGTTDASFGGEVMSYESPKPNIGIHRFIFVLFKQKRRQTVSVPSFRDHFNTRQFAVD}$ | 150 |
| 151 | NDLGLPVAAVYFNCQRETAARRR   | 173 |

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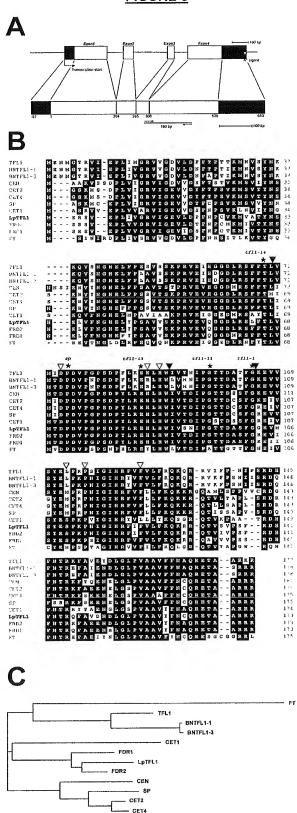
Inventor: Klaus K. NIELSEN et al.

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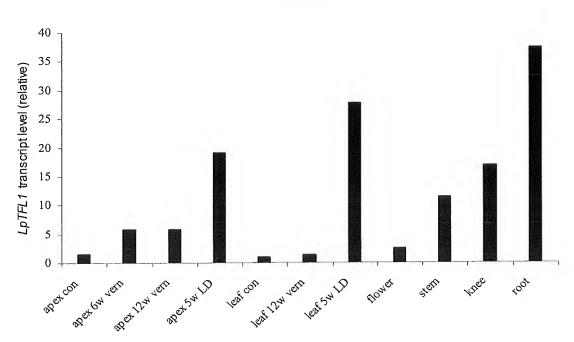
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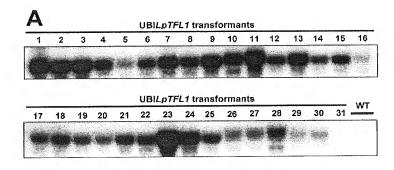
Docket No.: 0147-0262PUS1

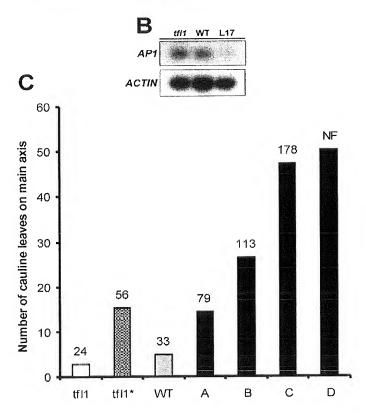
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REPLACEMENT SHEET

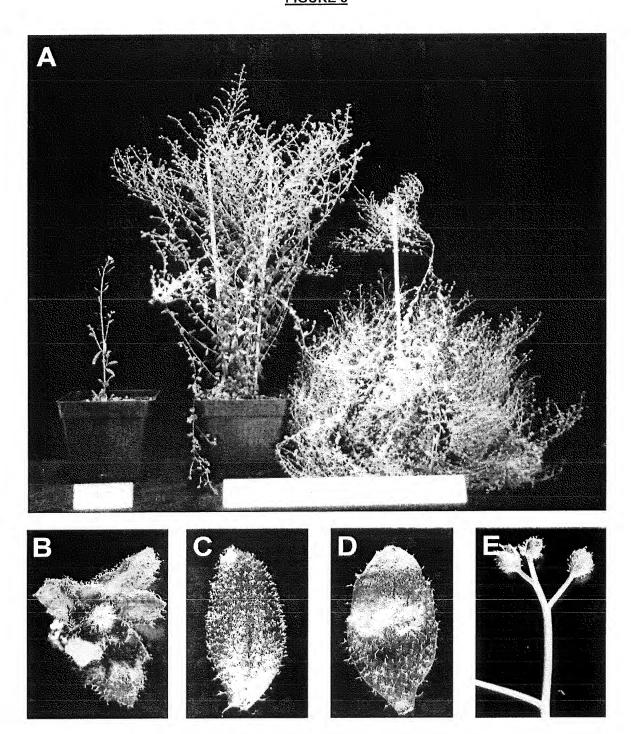
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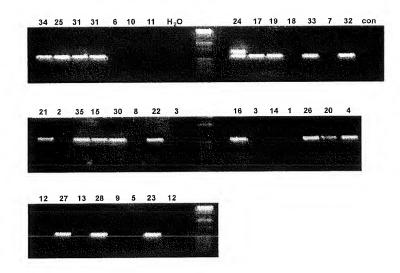
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# FIGURE 9



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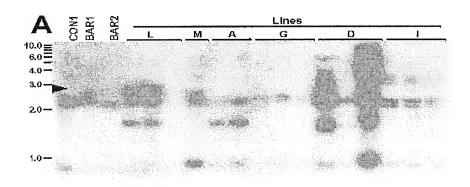
Docket No.: 0147-0262PUS1

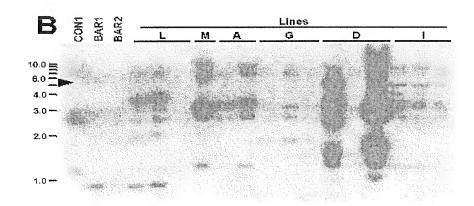
App No.: 10/507,355 Inventor: Klaus K. NIELSEN et al.

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CON1 CON2 BAR1

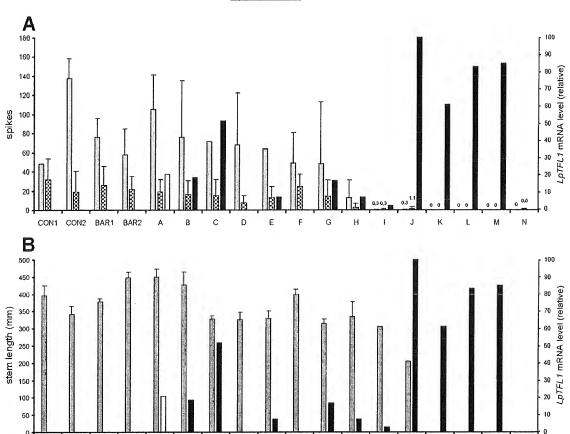
BAR2

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# FIGURE 12





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FIGURE 14

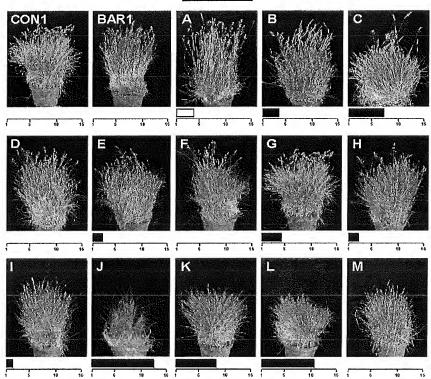
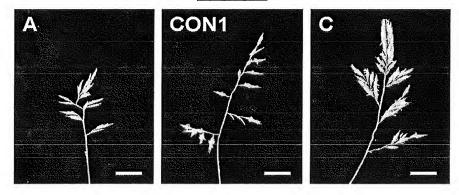


FIGURE 15



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| FIGURE 16: | Transformation | Efficiency and Floral | Activity of the | Transformants |
|------------|----------------|-----------------------|-----------------|---------------|
| Cultivar   | Line No.       | Inflorescences        | PCR             | RT-PCR        |
| F6         | CON            | 8                     | -               |               |
| F6         | 7              | 18                    | -               | -             |
| F6         | 8              | 11                    | _               | -             |
| F6         | 17             | 5,3                   | +               | -             |
| F6         | 18             | 13,3                  | +               | _             |
| F6         | 24             | 12                    | +               | +             |
| F6         | 29             | 0                     | +               | +             |
| F6         | 32             | 0                     | +               | +             |
| F6         | 33             | 4                     | +               | +             |
| F6         | 36             | 0                     | +               | +             |
| ACTION     | 2              | 1,8                   | _               | _             |
| ACTION     | 5              | 3                     | _               | _             |
| ACTION     | 9              | 0,3                   | _               | _             |
| ACTION     | 12             | 2                     | -               | <del>-</del>  |
| ACTION     | 13             | 0                     | -               | _             |
| ACTION     | 16             | 0                     | +               | <b>-</b>      |
| ACTION     | 19             | 7,3                   | +               | _             |
| ACTION     | 21             | 4                     | +               | +             |
| ACTION     | 22             | 0,3                   | +               | +             |
| ACTION     | 23             | 0                     | +               | +             |
| ACTION     | 25             | 0,3                   | +               | +             |
| ACTION     | 27             | 0                     | +               | +             |
| ACTION     | 28             | 4                     | +               | +             |
| ACTION     | 31             | 0                     | +               | +             |
| ACTION     | 34             | 0                     | +               | +             |
| ACTION     | 35             | 0                     | +               | +             |
| ΓELSTAR    | 1              | 10                    | -               | -             |
| TELSTAR    | 3              | 1                     | _               | -             |
| TELSTAR    | 4              | 11,6                  | _               | -             |
| TELSTAR    | 6              | 10,8                  | -               | -             |
| ΓELSTAR    | 10             | 5                     | -               | -             |
| ΓELSTAR    | 11             | 3,8                   | -               |               |
| ΓELSTAR    | 14             | 0                     | -               | -             |
| ΓELSTAR    | 15             | 3,8                   | +               | _             |
| ΓELSTAR    | 20             | 3,5                   | +               | <b></b>       |
| TELSTAR    | 26             | 0                     | +               | +             |
| TELSTAR    | 30             | 3,7                   | +               | +             |

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Figur 17: Transgene integration analysis by PCR using different primer combinations

| Primer combination                     |                    |         |                |    |    | 5             | 31::Lp1 | FLI tr         | UBI::LpTFLI transgenic lines <sup>a</sup> | c lines |               |    |     |    |            |                  |     |
|--|--------------------|---------|----------------|----|----|---------------|---------|----------------|---|---------|---------------|----|-----|----|------------|------------------|-----|
| CASSETTE UB promoter                   | intron Lanted WS   | CON BAR | A              | В  | C  | Ω             | Щ       | ĹĽ             | Ŋ   | H       | _             | 'n | ∠ ∠ | 1  | M          | z                | ۵   |
| MS33-LP575                             |                    |         |                |    |    | 0.8           |         |                |   |         |               | +  |     | +  |            | 0.8              | 2.3 |
| MS33-LP4REV                            |                    |         |                |    |    | 0.55          |         |                |   |         |               | +  |     | +  |            | 0.55             | 2.0 |
| MS31-LP4REV                            |                    |         | +              |    |    | +             |         |                | 1.4                                       | +       |               | +  |     | +  | +          |                  | 1.5 |
| MS56-LP575                             |                    |         |                | +  | +  | +/0.5         | +       |                | +/0.5                                     | +       | +             | +  | +   | +  | +          | +/0.5            | 9.0 |
| LP0-MS8                                |                    |         |                | +  | +  | W/+           | +       |                | +   | +       | +/1.8         | +  | +   | +  | +          | +/1.6            | 9.0 |
| MS56-LP4REV<br>(intron::Lp7Ff.1 probe) |                    |         | +              | +  | +  | +             | +       | +              | +   | +       | +             | +  | +   | +  | +          | +                | 0.4 |
|  | Promoter           |         | short          | 1  |    | TATA          |         |                | short short                               | short   |               | ķ  |     | oķ | short TATA | -<br>IATA<br>box |     |
| Result                                 | Intron             |         | ķ              |    |    | 상             |         |                | 상   | ok      |               | ok |     | 상  | 성          |                  |     |
|  | <i>LpTFL1</i> cDNA | 1 0     | trun-<br>cated | øk | ok | ok +<br>extra | Ą       | trun-<br>cated | ok  | 쓩       | ok +<br>extra | 쓩  | ķ   | ķ  | ø          | ok +<br>extra    |     |

\*plus indicates that the observed fragment had the expected size, whereas numbers indicate that the fragment size deviated from the expected size (numbers in bold), blank field indicates that no PCR-product was detected; E, EcoRJ; H, HindIII